

**Parthenogenetic *Ischnura hastata* revisited:
present status and notes on population ecology and behaviour
(Odonata: Coenagrionidae)**

**M. Olalla Lorenzo-Carballa¹, Christopher D. Beatty¹, Carlo Utzeri²,
Virgilio Vieira³ & Adolfo Cordero-Rivera^{1,4}**

¹Grupo de Ecología Evolutiva e da Conservación, Universidade de Vigo, EUET Forestal,
Campus Universitario, 36005 Pontevedra, Galiza, Spain.

<olalla.lorenzo@uvigo.es>, <beatty@ch@yahoo.com>, <adolfo.cordero@uvigo.es>

²Dipartimento di Biologia Animale e dell'Uomo, Viale dell'Università 32, 00191 Roma,
Italy. <carlo.utzeri@uniroma1.it>

³Departamento de Biologia e CERN, Universidade dos Açores, Apartado 1422, 9501-801
Ponta Delgada, Açores, Portugal. <vvieira@uac.pt>

⁴Author for correspondence

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ABSTRACT

Populations of *Ischnura hastata* found in the Azores archipelago represent the only known example of parthenogenesis in the order Odonata. In this paper, we present results from fieldwork done on the islands of São Miguel, Pico, Santa Maria, and Graciosa, aimed at characterizing population ecology and habitat preferences of this species. Sampling of several ponds in the islands of São Miguel and Pico showed that *I. hastata* occurred in oligotrophic ponds, but was absent from all eutrophic ponds sampled, many of which have been impacted by cattle grazing and water extraction by humans. This suggests that parthenogenetic populations are highly sensitive to eutrophication, which may be different from suggested habitat preferences of sexual populations for this species. Mark-recapture studies showed *I. hastata* to occur in high densities in the studied populations. Although life expectancy of mature females was estimated at less than one week, their high fecundity and fertility could potentially explain the large number of individuals observed in some of the studied sites. Submerged oviposition seems to be a common behaviour, probably evolved as an adaptation to unfavourable climatic conditions and to avoid egg desiccation caused by water depletion. In summary, this work represents a first attempt to study the ecology and population biology of parthenogenetic populations of *I. hastata*, and may help us to understand the unique conditions under which these populations could have evolved and how to best insure their conservation.

INTRODUCTION

The Azores are a remote oceanic archipelago of nine volcanic islands. Terrestrial organisms native to these islands have arrived through a number of mechanisms: transport by wind, ocean currents, other animals, or by human activity, either through purposeful or accidental introduction. Currently, there are approximately 4,500 species of terrestrial fauna and flora listed for this archipelago, a low species diversity compared with that of the Canary Islands, which is at least two-thirds higher (Borges et al. 2005). This is likely due to the great distance of the Azores Islands from either the Old or New Worlds, both of which have served as sources of colonization for the Azores. For example, Santa Maria, the easternmost island in the archipelago, is ca 1,400 km from Portugal, and Corvo, the westernmost island, is ca 1,800 km from Terra Nova, Canada (Constância et al. 2001).

Only four odonate species are known from the Azores (Valle 1940; Gardner 1959, 1960; Belle 1992; Cordero Rivera et al. 2005). Three of these – *Ischnura pumilio* (Charpentier), *Anax imperator* Leach, and *Sympetrum fonscolombii* (Selys) – belong to the European fauna, whereas the fourth, *Ischnura hastata* (Say), is native to the Americas (Belle & van Tol 1990). Although other possible cases exist in Fiji (Beatty et al. 2007; Van Gossum et al. 2007), and with the exception of the artificial parthenogenesis reported for the species *Stylurus annulatus* (Djakonov) and *S. oculatus* (Asahina) (Kato et al. 1997; Watanabe et al. 1999), *I. hastata* populations found in the Azores constitute the only known example of natural parthenogenesis in the order Odonata (Cordero Rivera et al. 2005; Lorenzo-Carballa & Cordero-Rivera 2009), which gives this place a great relevance from an evolutionary and conservation point of view.

Despite the widespread distribution of *I. hastata* in the American continent, spanning from Canada to Ecuador (Westfall & May 1996), very little is known about the biology and behaviour of its sexual populations. To our knowledge, there are no published records on the reproductive behaviour of this species, although Robinson & Allgeyer (1996) have proposed it to be monogamous based on reported mating frequencies of mature females, demographics, and behaviour. Furthermore, almost no information exists on its population ecology, except for anecdotal accounts on its ability to disperse and colonise new habitats, for which it has been considered a migratory species (see Corbet 1999: 407, 411, 649 and references therein). Published studies including this species have analysed phylogenetic relationships with other *Ischnura* species (Chippindale et al. 1999), tolerance to pollution (Osborn 2005), as well as interactions with mite parasites (Lajeunesse 2008).

In a previous paper we reviewed the literature records of odonates from the Azores, and provided a list of localities sampled by some of us in seven of the nine islands of the archipelago during 2000 and 2003 (Cordero Rivera et al. 2005). The results of that work indicated that parthenogenetic *I. hastata* was widespread, and we recorded it in six of these islands. Nevertheless, we noted that these populations were apparently absent from some ponds, and recorded a recent extinction in the Lagoa do Capitão on Pico.

In the present paper we summarize the results of field work done on the islands of São Miguel, Pico, Graciosa, and Santa Maria, aimed at characterizing the habitat preferences and population ecology of parthenogenetic *I. hastata* (Pl. VIIIa).

METHODS

The distribution of *Ischnura hastata* in relation to habitat parameters

Odonate surveys, focusing mainly on *I. hastata*, were conducted by VV at Graciosa Island in June 2004 and August 2007; the island of Santa Maria was visited in August 2008 by ACR, CU, and VV. These were the only two islands not sampled by Cordero et al. (2005). Further field work was done at Pico in July and August 2008 by ACR and CB. Many ponds at São Miguel were periodically sampled by VV from 2006-2008 and population studies (see above) were conducted in July-August 2008 at several ponds by OLC, ACR, and CU.

In a previous paper we suggested that *I. hastata* was apparently absent from eutrophic lakes and ponds (Cordero Rivera et al. 2005), many of which were impacted by cattle grazing or water removal. This led us to estimate the presence and abundance of the species in detail in São Miguel and Pico, to test whether an association exists between habitat quality and presence/abundance of *I. hastata*. To this end, we visited 10 ponds in Pico and 17 ponds in São Miguel between July and August of 2007 and 2008 (Table 1). Names of water bodies in Pico follow Gonçalves et al. (2006b).

Many of the ponds in the Azores which *I. hastata* inhabited were found at high elevations (average 640 m a.s.l., Cordero Rivera et al. 2005), and were often shrouded in fog or light rain. For distribution and density studies, collection of adults was carried out when meteorological conditions were favourable; i.e., when the weather was sufficiently sunny and warm for adult females to fly. Individuals were collected using a hand-held aerial net, sweeping vegetation immediately adjoining the pond, where females were often encountered. After sweeping, individual females were removed quickly from the net and a unique number was placed on their wings with a fine-point indelible pen (STABILO®OHPen-S). Marking each individual prevented us from counting the same individual more than once, and also allowed for an estimation of adult survivorship over several days. In some of the ponds, all captured individuals were marked.

Trophic categories – oligotrophic, mesotrophic, or eutrophic – were assigned to each sampled pond by independent sources (P. Raposeiro pers. comm.), based on a number of water quality parameters measured in these ponds (pH, temperature, conductivity, turbidity, planktonic and benthic community composition: Gonçalves et al. 2005, 2006a, b; P. Raposeiro unpubl. data). Assessments of the trophic levels of our study ponds were performed by P. Raposeiro without knowledge of the objectives of our study, or of the distributions of *I. hastata*. Two of our study ponds, Lagoa do Cabeço da Lagoinha (Pico) and Lagoa do Pico da Lagoa (São Miguel) were not assessed in these studies, and we classified both of these ponds as oligotrophic, given the clarity of the water and the presence of emergent shoreline vegetation, which is associated with increased levels of dissolved oxygen (Osborn 2005).

Mark-recapture and behavioural observations

Ecological and behavioural studies on *I. hastata* were conducted on the islands of São Miguel and Pico in July and August 2008. Mark-recapture studies in São Miguel were conducted at the Lagoa do Areeiro, over 16 days from 27 July to 24 August 2008. This was an almost circular pond (Fig. 1a), with a maximum size of 97 x 77 m

and 2.7 m of maximum depth, that occupied 0.03 km² of catchment (Constância et al. 2001). It was located inside a small caldera, and surrounded mainly by plantations of *Cryptomeria japonica* and some remnants of native laurel forest, which constituted the habitat of a variety of bird taxa, including *Fringilla coelebs moreletti* and *Turdus merula azorensis* (Constância et al. 2001). It was a fishless pond, and had a low density of frogs (*Rana perezi*, an introduced species). Shore vegetation, on



Figure 1: Two examples of optimal habitats for *Ischnura hastata* in the Azores islands — (a) Lagoa do Areeiro, São Miguel; (b) Lagoa do Gato, Pico. Note the presence of abundant vegetation in the second case, which covers almost 100% of the water surface. Photos by ACR.

which zygopterans are highly dependent for feeding and escaping from predators (Corbet 1999: 9-20), was well preserved at this pond because there was no access to cattle and restricted access to humans, due to its relative isolation and the difficulties of reaching the crater. Aquatic vegetation included some small masses of *Nymphaea alba* and *Potamogeton polygonifolius* (Constância et al. 2001).

In Pico Island, work was done at two ponds: Lagoa do Cabeço do Caveiro and Lagoa do Gato, from 15 to 19 August 2008. The first was a circular pond in a crater, and the second a topographic depression that accumulates water (Gonçalves et al. 2006b). Both had large masses of *P. polygonifolius*, which in the latter pond covered almost all the surface (Fig. 1b). Cattle have been observed accessing both ponds, but much of the shoreline vegetation remained intact, suggesting that the cattle had only a moderate-to-low impact on vegetation. The perimeter of these ponds was estimated from the map of the 'Instituto Geográfico do Exército' (Lajes do Pico, sheet 12, scale 1:25,000). The map was scanned and the perimeter estimated with ImageTool 3.0 software (available at: <<http://ddsdx.uthscsa.edu/dig/itdesc.html>>).

I. hastata adults were captured with hand nets and marked. Individuals of *I. pumilio* were also marked during the study, but their abundance in these populations was so low that no estimates of population parameters could be obtained.

Data on mark-recapture histories were analyzed using the full time-dependent Cormack-Jolly-Seber (CJS) model implemented in POPAN5 (Arnason et al. 1998) to obtain population size estimates. To estimate survival rate we used the information-theory approach implemented in MARK 5.1 (Cooch & White 2007), which is based on the Akaike Information Criterion (AIC) (Burnham & Anderson 1998). Analysis of recapture histories was modelled with two female age groups (immature and mature). We first explored the adjustment of a full age (a) and time-dependent (t) model to the data, $\Phi(a*t) p(a*t)$ (Lebreton et al. 1992). We estimated the overdispersion parameter ($c\text{-hat}$) from the bootstrap procedure in MARK, and this value was used to correct parameter estimates.

Focal observations on reproductive behaviour of parthenogenetic females were conducted by walking along the shore to detect oviposition substrates and possible interactions with males of *I. pumilio*.

An automatic data logger (Tinytag Gemini Data Logger) was placed in the Lagoa do Areiro, in order to monitor air temperature during the study period. The logger was placed ca 30 cm from the soil surface, in an area where most females of *I. hastata* were concentrated during the study. We also recorded water temperature by placing a probe at a depth of ca 10 cm, approximately the same depth where females laid eggs. Due to unpredictable water level changes, this probe remained out of the water for a short period of time, between 17 and 24 August.

Egg activation, fecundity, and fertility

The genital tract of Odonata includes a vagina with a fertilization region and two sperm storage organs: the bursa copulatrix and the spermatheca. After copulation, eggs are fertilized (activated) as they pass through the oviduct (Corbet 1999: 508-509). To test if oviposition is needed for the activation of the eggs in parthenogenetic *I. hastata*, mature eggs were extracted from the ovaries of six females and placed in water to follow their development. If unfertilized eggs require passage through the oviduct for activation to occur, one would expect these eggs extracted from the ovaries of parthenogenetic females not to develop.

To obtain estimates of fertility of parthenogenetic females in the field, and thus complement the results of Lorenzo Carballa & Cordero Rivera (2007), 33 mature females were captured at five different ponds in São Miguel Island: Lagoa do Fogo, L. do Areeiro, L. do Carvão, L. do Pico da Lagoa, and L. do Pau Pique. One clutch was obtained from each female as described in Lorenzo Carballa & Cordero Rivera (2007).

After the eggs hatched, clutches were preserved in absolute ethanol and eggs were counted under a binocular microscope. Fertility was determined by scoring the percentage of eggs that hatched. Dark eye spots are visible in the embryo after 12 days; if no such eye spot was found in a non-hatched egg, the egg was scored as sterile (Fincke 1984).

RESULTS

Distribution of *Ischnura hastata* in relation to habitat parameters

I. hastata was rare in Santa Maria, with only two females found in the Ribeira do Sancho (UTM 26S 664852, 4090617) in July 2008. Despite intensive searches in Lagoeiro do Barreiro and Lagoeiro do Tanque, the two water bodies on Graciosa, VV was unable to find *I. hastata* in this island.

Table 1 presents the distribution data of *I. hastata* and *I. pumilio* in the sampled ponds in Pico and São Miguel. We scored abundance of the species collecting all the individuals found along the shore line during 20 min to 1 h. Sometimes we performed non-quantitative sampling. In these cases we indicated only presence/absence of each species or an index of abundance, with 'common' indicating that the species was easily found in large numbers, at least 20 individuals in a few minutes, and 'rare' meaning that only one to five individuals were found. Results of our sampling indicate that an inverse relationship exists between water pollution and the presence of *I. hastata*, with this species being dominant in oligotrophic ponds, and absent in all eutrophic ponds sampled. A contingency chi-square table was constructed with data from Table 1, scoring the abundance of *I. hastata* over the three trophic categories (oligo-, meso-, and eutrophic). Thus, *I. hastata* was common, i.e. the dominant damselfly, in all nine oligotrophic and five out of 11 mesotrophic ponds; it was present but not the most common species in only four mesotrophic ponds, and absent from two meso- and all seven eutrophic ponds. We considered it dominant in Lagoa do Gato and Pau Pique although it was not the most common species in the first sampling. Results of the contingency analysis indicated a significant relationship between the trophic state of the pond and the abundance of *I. hastata* (adjusted $\chi^2 = 26.7$, d.f. = 4, $p < 0.001$). This relationship was not detected in the case of *I. pumilio* (adjusted $\chi^2 = 4.00$, d.f. = 4, $p = 0.406$).

Population density

Lagoa do Areeiro (São Miguel): We marked 2,752 females of *I. hastata* and recaptured 983 at least once. Ten females and 14 males of *I. pumilio* were marked without recaptures. The size of this *I. hastata* population was estimated at ca 2,000 individuals during most of the study period, but peaked at 4,800 on 29 July (Fig. 2). Estimates have low standard errors due to moderately high recapture rates (up to 6x

per individual). Mean density was therefore ca 7.0 females per meter of shore line, considering a mean diameter of 90 m. This estimate matches our observations during field work.

Lagoa do Cabeço do Caveiro (Pico): We marked 423 females of *I. hastata*, but only 40 were recaptured one or two times. Only one female of *I. pumilio* was observed. Population size estimates indicate an average of 610 individuals over the study period (Fig. 2), but with large standard errors. Density was ca 2.8 females per meter of shore line, estimating the pond perimeter as 220 m.

Lagoa do Gato (Pico): In this pond we marked 619 females of *I. hastata*, and recaptured 57 one or two times. Of 27 individuals of *I. pumilio*, only two were recaptured. Population size estimates indicate an average of 1,440 individuals of *I. hastata* over the study period, excluding the last estimate (18 August) which has a large error (Fig. 2). Density was ca 4.1 females per meter of shore line, estimating the perimeter as 350 m.

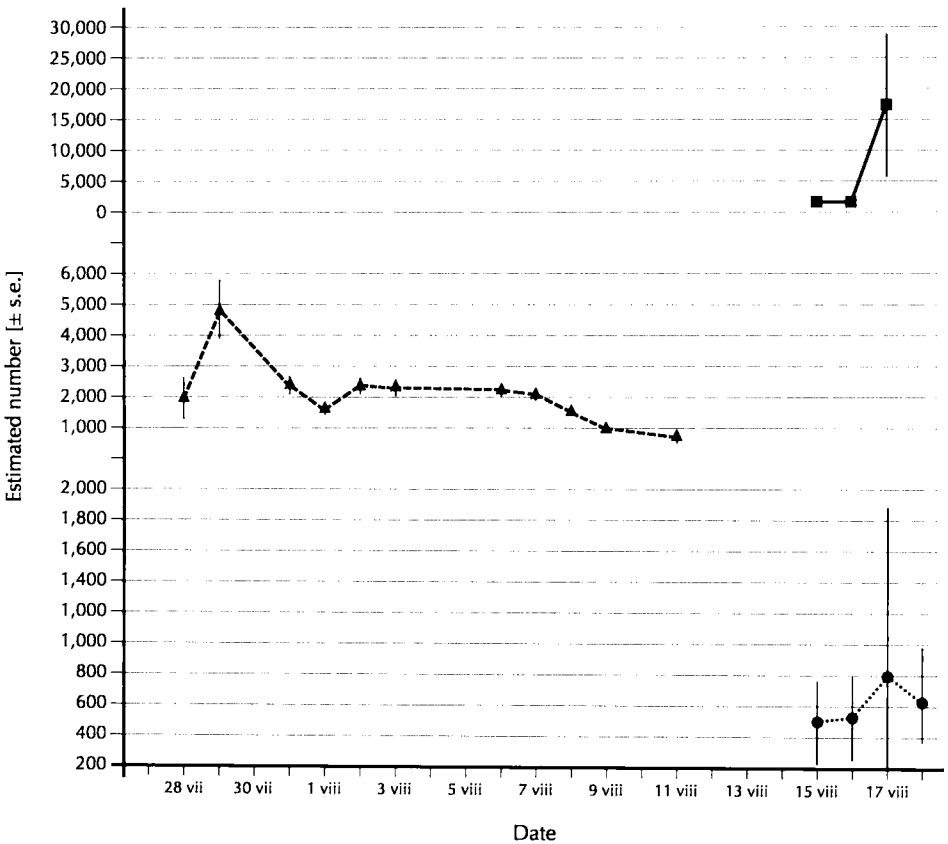


Figure 2: Population size estimates and s.e. of *Ischnura hastata* at different ponds in the Azores — (▲) Lagoa do Areeiro, São Miguel; (●) Lagoa do Cabeço do Caveiro, Pico; (■) Lagoa do Gato, Pico. Data were analyzed using the CJS full-time dependent model.

Table 1. The status of *Ischnura hastata* and *I. pumilio* in selected ponds at Pico and São Miguel. The numbers indicate the abundance of each species when sampling was quantitative and presence/abundance in categories otherwise (see text for more details). The trophic state of each pond was obtained from Gonçalves et al. (2005, 2006a, b) and personal communication with a local scientist (P. Raposeiro pers. comm.), except for Lagoa do Cabeço da Lagoinha and Lagoa do Pico da Lagoa, which were classified by us as oligotrophic. O: oligotrophic; M: mesotrophic; E: eutrophic.

Pond	Trophic state	Coordinates Date	<i>hastata</i> ♀	<i>pumilio</i> ♂/♀	<i>hastata</i> [%]
Pico Island					
Lagoa do Cabeço da Lagoinha	O	38°25'21.9"N, 28°7'22.71"W 23 vii 2008	83	0/0	100.0
Lagoa do Cabeço do Caveiro	O	38°26'7.27"N, 28°11'47.47"W 14-19 viii 2008	423	0/1	99.8
Lagoa do Cabeço Verde	O	38°26'6.35"N, 28°10'11.8"W 24 vii 2008	32	11/5	66.7
Lagoa do Caiado	M	38°27'21.4"N, 28°15'8.5"W 23 vii 2008	4	52/26	4.9
Lagoa do Capitão	E	38°29'14." N, 28°19'1.9"W 22-23 vii 2008	-	Absent	Absent
Lagoa do Gato	O	38°26'6.9"N, 28°10'11.2"W 24 vii 2008	27	44/24	28.4
	O	15-19 viii 2008	619	17/10	95.8
Lagoa do Landroal*	O	38°27'28.3"N, 28°16'4.2"W 22-23 vii 2008	134	23/15	77.9
Lagoinha do Paul	O	38°25'46.7"N, 28°6'17.4"W 22 vii 2008	63	1/0	98.4
Lagoa do Peixinho	E	38°26'3.0"N, 28°10'22.6"W 24 vii 2008	-	Absent	Absent
Lagoa da Rosada	E	38°26'6.3"N, 28°11'17.7"W 24 vii 2008	-	Absent	Absent
São Miguel Island					
Lagoa das Achadas	M	37°48'56.7"N, 25°43'52.7"W 13 vii 2008	-	Rare	Rare
Lagoa das Canas	M	37°48'45.8"N, 25°43'37.0"W 11 vii 2008	37	8/3	77.1
Lagoa das Empadadas (South)	M	37°49'26.9"N, 25°44'50.1"W 13 vii 2008	-	Absent	Common
Lagoa do Congro	E	37°45'13.8"N, 25°24'20.3"W vii 2008	-	Absent	Absent
Lagoa das Furnas	E	37°45'52.1"N, 25°19'39.6"W 2005-2007, vii 2008	-	Present	Absent
Lagoa das Sete Cidades	E	37°51'18.9"N, 25°47'11.5"W 2005-2007, vii 2008	-	Present	Absent
Lagoa de São Brás	E	37°47'30.3"N, 25°24'33.2"W 27 vii 2008	0	85/27	0.0
	E	03 viii 2008	0	47/20	0.0
	E	19 viii 2008	0	79/31	0.0
Lagoa do Areeiro	O	37°45'48.0"N, 25°25'35.7"W 14 vii 2008	40	1/0	97.6
	O	27 vii-24 viii 2008	2,752	10/14	99.1
Lagoa do Canário	M	37°50'8.1"N, 25°45'36.5"W 23 viii 2007, viii 2008	-	Common	Present

Pond	Trophic state	Coordinates	<i>hastata</i> ♀	<i>pumilio</i> ♂/♀	<i>hastata</i> [%]
		Date			
Lagoa do Carvão		37°49'33.7"N, 25°44'27.5"W			
	M	08-09 vii 2008	95	3/1	96.0
	M	02 viii 2008	64	5/3	88.9
Lagoa do Fogo		37°46'6.4"N, 25°28'54.3"W			
	M	21 vii 2008	-	Rare	Rare
Lagoa do Pau Pique		37°49'53.8"N, 25°44'43.0"W			
	M	11 vii 2008	3	6/7	18.8
	M	02 viii 2008	62	1/2	95.4
	M	15-16 viii 2008	276	3/1	98.6
Lagoa do Peixe		37°49'7.9"N, 25°44'10.0"W			
	M	2005-2007, vii 2008	-	Present	Absent
Lagoa do Pico da Lagoa		37°46'8.5"N, 25°24'10.1"W			
	O	14 vii 2008	68	0/0	100.0
Lagoa do Pico de El Rei		37°46'24.6"N, 25°23'11.2"W			
	M	12 vii 2008	-	Absent	Absent
Lagoa Rasa (Serra Devassa)		37°49'28.1"N, 25°45'5.5"W			
	M	23 viii 2007	-	Rare	Common
Lagoa Rasa (Sete Cidades)		37°50'38.0"N, 25°46'45.1"W			
	O	25 vii 2008	30	22/5	52.6

*referred to as Lagoa do Cabeço da Rocha in Cordero Rivera et al. (2005)

Survivorship

Given the low number of recaptures obtained in the populations from Pico, analysis of survivorship was done only with the data from Lagoa do Areeiro. The goodness-of-fit tests implemented in Mark indicate that TEST 2, which is useful for testing the basic assumption of "equal catchability" of marked animals is significant ($\chi^2 = 85.1$, d.f. = 63, $p = 0.0331$). TEST 3, which tests the assumption that all marked animals alive at day (i) have the same probability of surviving to day ($i+1$), was not significant ($\chi^2 = 39.2$, d.f. = 40, $p = 0.508$). This means that females marked as immature (including teneral) and those marked as mature have different recapture rates, and that some heterogeneity exists in these groups, but this does not affect survival. The overall TEST 2+TEST 3 was marginally significant ($\chi^2 = 124.3$, d.f. = 103, $p = 0.075$). We explored all possible models (16) to select the best using the AIC, although we were a priori interested in differences among age groups in survival and the effect of time on this parameter. Results indicate that the model with the lowest corrected AIC value (AIC_c) is $\Phi(t) p(a^*t)$, i.e., that female survival rate is affected by time but not by age, and that recapture rate was time and age dependent, with an interaction between both factors. This indicates that female survival rate was variable over the study period. This model is highly supported by the data ($QAIC_c$ weight = 0.997). The second model ($QAIC_c = 13.250$) is the model $\Phi(a) p(a^*t)$, i.e., which includes age effects on survival and age and time effects on recapture rates, with an interaction between both factors. This model suggests that mature females had a 2% lower probability of being recaptured (0.855 for immature and 0.829 for mature females), but is not well supported by the data ($QAIC_c$ weight = 0.001). Average survival rate for all females over all days was 0.840 (s.e. = 0.006; 95% CI = 0.826-0.852), and mean recapture rate 0.173 (s.e. = 0.006, 95% CI = 0.160-0.186), both derived from the most reduced model $\Phi(.) p(.)$.

Table 2. Fertility of eggs dissected from parthenogenetic females and incubated in water.

Female	# extracted eggs	# emerged larvae	Fertility [%]
1	43	36	83.7
2	78	73	93.6
3*	34	15	44.1
4	69	66	95.7
5	49	45	91.8
6	7	7	100.0

*young female.

Behavioural observations

Parthenogenetic *I. hastata* females at Lagoa do Areeiro showed little activity, probably due to low air temperatures and cloudy conditions that were common during the study period (Fig. 3). Females were found among the shore vegetation at some distance from water and remained perched most of the time, with the exception of short flights for foraging. Despite the high density of individuals at this pond (see above), only 24 ovipositing females were recorded over a total of 48 h of field work. All ovipositions were observed between 09:40 and 15:40 h (solar time). Six of these females completely submerged to oviposit (Pl. VIIIb), whereas five were observed attached to emerging vegetation, probably drying after resurfacing. In all six cases in which females were observed ovipositing under water, they first laid a few eggs in the plant stems or leaves over the water and they then started moving down the stems until they were completely submerged (Pl. VIII). The rest of the observed females (13) oviposited on the surface vegetation, mainly on leaves of *Potamogeton polygonifolius*, usually with the abdomen completely submerged. One female was observed in a short-duration tandem with a male of *I. pumilio*.

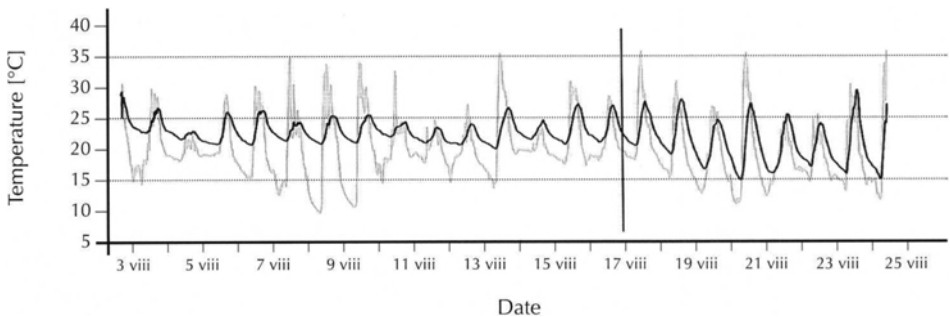


Figure 3: Air and water temperatures recorded in Lagoa do Areeiro, São Miguel, during August 2008. Note that water temperature (black) was higher than air temperature (grey) variation during the night and the early hours of the morning. The increase in water temperature observed after 17 August as indicated by the vertical bar was due to a water level drop, after which the probe remained completely out of the water.

Table 3. Fecundity and fertility of 33 parthenogenetic *I. hastata* females collected at five different ponds in São Miguel Island, in July and August 2008.

Population	Day of oviposition	# fertile eggs	Total # eggs	Fertility [%]
Lagoa do Carvão	08 vii	19	19	100.0
		35	36	97.2
		31	32	96.9
	09 vii	69	70	98.6
		55	56	98.2
		75	76	98.7
		109	112	97.3
Lagoa do Pico da Lagoa	14 vii	70	70	100.0
		58	60	96.7
		97	100	97.0
		96	97	99.0
		54	54	100.0
Lagoa do Fogo	21 vii	215	219	98.2
Lagoa do Areeiro	17 vii	59	59	100.0
		56	56	100.0
		127	131	97.0
		104	105	99.1
		106	111	95.5
	13 viii	30	38	79.0
		66	66	100.0
		66	69	95.7
		31	32	96.9
		182	183	99.5
		33	36	91.7
		79	80	98.8
		16	16	100.0
Lagoa do Pau Pique	16 viii	73	76	96.1
		46	47	97.9
		53	55	96.4
		78	78	100.0
		25	25	100.0
		76	79	96.2
		33	35	94.3
		Mean	72.1	97.3
		s.e.	7.8	0.7

Egg activation, fecundity, and fertility

In five out of six females, most of the eggs extracted from the ovaries and incubated in water underwent normal development and hatched (average fertility = $93.0 \pm 2.7\%$, $n = 5$; Table 2). One young female was an exception to this trend, but even in this case 44% of the eggs were fertile. Number of larvae obtained ranged from seven to 73 per female (Table 2). Larvae from two of these females were reared to adulthood (Pl. VIIIc, d), and reproduced successfully as adults. The developmental stages of the eggs extracted from ovaries are shown in Figure 4.

Clutch size from females collected in the field was 72.1 ± 7.6 eggs on average ($n = 33$ females, range 16-219 eggs, Table 3). Fertility rate was $97.3 \pm 0.7\%$ (range 79-100%, Table 3).

DISCUSSION

Population size, demography, and behaviour

Results of mark-recapture studies indicate that population densities of parthenogenetic *Ischnura hastata* colonies can be very high. In favourable habitats, like Lagoa do Areeiro (Fig. 1a) estimates of density ranged between 5-10 females per meter of shoreline. The estimates of daily survival rate in this population (0.84) suggest that females of *I. hastata* survive on average less than one week (5.74 days). Therefore, this population must produce a large number of adults in order to maintain these high densities, which translates into huge population sizes throughout the season. Given the daily estimates obtained for some ponds (Fig. 2), and assuming that oligo- and mesotrophic ponds are the best habitats for this species (see Table 1), these might produce well over 10^5 animals per year, which ensures the persistence of these parthenogenetic populations in the Azores, at least in the short term.

Given the low number of ovipositions observed during the study period, these high-density populations could be explained by a relatively high survivorship, combined with the high fertility observed in this species. The mean fertility rate of females collected in the field (97.3%; Table 3) is even higher than estimates for parthenogenetic females reared in the laboratory (93.9%; Lorenzo Carballa & Cordero Rivera 2007). Also, the paucity of competitors and predators on the archipelago could account for the high number of individuals observed in some populations: as pointed out in the introduction, there are only four odonate species in the archipelago, and the results of our sampling show that both zygopterans present in the islands (*I. hastata* and *I. pumilio*) rarely coexist at the same pond (see Table 1). While competitive exclusion could have some influence for this lack of overlap, with one species preventing the other from establishing, the correlation of the distribution of these two species with different habitat types indicates that they have different habitat affinities. Furthermore, in many ponds there are no fishes and almost no frogs because of low pH, this being the case for Lagoa do Areeiro for example (Constância et al. 2001). These factors could reduce mortality during the larval stage and thus contribute to an increase in the number of adults finally emerging in these populations.

The low activity recorded for *I. hastata* females in the studied populations could be due to windy conditions and rain, which were common during our field work period, especially at high altitudes where we found most of the *I. hastata* populations, although similar observations have been documented for the sexual form of this species (Dunkle 1990). Perhaps one of the most interesting findings of this study was that underwater oviposition is relatively common. Although in the Pau Pique pond all females laid eggs on floating vegetation, at least one female was seen laying eggs underwater in Pico (Cordero Rivera et al. 2005: Plate VIII), which suggests that this could also be an oviposition strategy in populations of other islands.

Completely submerged oviposition has been reported for many species of Zygoptera in different families and also for a few Anisoptera in the family Aeshnidae, although it is an infrequent phenomenon in *Ischnura* (see Corbet 1999: 30-32 and references therein; and also Hawking et al. 2004 for underwater oviposition in Anisoptera). Females showing this behaviour are subjected to the risk of predation by a variety of predators including large anisopteran larvae, Hemiptera, Coleoptera, fishes, and frogs (Corbet 1999: 33). An additional risk of submerged oviposition results from the impossibility of some females of resurfacing: Miller (1990) recorded

that 27% of *Enallagma cyathigerum* (Charpentier) ovipositing underwater were abandoned by their mates and probably drowned, whereas ca 3% of the females of *E. hageni* (Walsh) studied by Fincke (1986) died because they were unable to resurface.

In the case of parthenogenetic *I. hastata*, submerged oviposition could also be contributing to the maintenance of high population densities. We found evidence for direct egg development without oviposition (Fig. 4), since some larvae were able to eclose from the abdomen when a female was killed and left on the water, or if the abdomen was severed simulating a predator attack (unpubl.). This opens up the possibility that, if females die because they drown or are unable to resurface, or if part of their bodies are eaten by predators, some eggs could still be able to hatch.

In sexually reproducing females, submerged oviposition serves as an escape from male harassment (Miller 1990, 1994; Corbet 1999: 32). In our case, this cannot be claimed as the explanation for this behaviour in *I. hastata*, because although males of *I. pumilio* sometimes take female *I. hastata* in tandem, we have shown that in most cases *I. pumilio* is scarce where *I. hastata* is common (Table 1). The scarcity of aquatic vegetation in Areeiro (Fig. 1a), might explain this behaviour, but this cannot be a general explanation because large masses of floating *Potamogeton* leaves are common in the most favourable habitats for *I. hastata*, where this behaviour has also been observed (Fig. 1b). Density of above-water and underwater predators could also influence this behaviour, and explain why in some ponds like Areeiro, with a high density of insectivorous birds but no frogs or fishes in the water (see Methods), most of the observed ovipositions occur under water, whereas in Pau Pique, where the situation is the opposite (high density of predators in water; Constância 2001), all the females observed laid eggs on the surface.

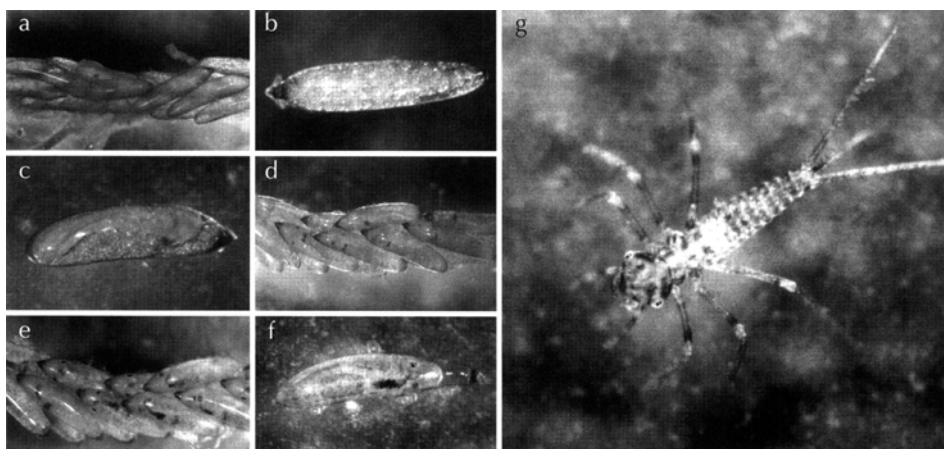


Figure 4: Direct development of eggs extracted from the ovaries of parthenogenetic *Ischnura hastata* and incubated in water — (a) at day 0; (b) at day 3, the segmented embryo can be distinguished; (c, d) at day 9, embryos are clearly visible inside the eggs; (e) at day 13, the eye spots become visible; (f, g) at day 15, eggs start to hatch (f) and first-instar larvae (g) appear. Photos by ACR.

The major reason for underwater oviposition in *I. hastata* is likely to be due to the climatic conditions that predominate in the Azores; in our study site, air temperature oscillations, with approximately a 20°C difference between maxima and minima in some days, contrasted with more constant underwater temperatures (Fig. 3). Wind and waves at water surface, causing interruptions of oviposition, were also frequent in the studied ponds, as well as changes in water level, which could influence this behaviour. For example, water levels were 1.5 m lower in the Lagoa do Areeiro at the end of our study period, due mainly to water extraction by local farmers, thus leaving most of the places used by females to oviposit exposed. By ovipositing underwater, females of *I. hastata* might thus decrease the chance of egg desiccation due to changes in water level (Fincke 1986). This could also be especially important in temporary ponds, which constitute a common habitat for *I. hastata* in North America (OLC pers. obs.). Further research is needed to evaluate the extent and the causes of this behaviour in this species, both in sexual and parthenogenetic populations.

Effects of habitat degradation on *I. hastata*

Our sampling results on the islands of São Miguel and Pico suggest that parthenogenetic *I. hastata* populations are unable to survive in degraded lakes and ponds. We have found that *I. hastata* is the dominant zygopteran in oligotrophic conditions, and may also persist in large numbers in mesotrophic ponds, but we never recorded it in eutrophic ponds, even in some cases where we also searched for larvae. Many of these eutrophic ponds have been affected by cattle, which trample streamside vegetation and increase organic inputs and turbidity. Water is also removed by local farmers for transport to cattle watering tanks. Through these alterations, shallow margins of ponds are degraded and water quality is affected. *I. pumilio* dominates some eutrophic lakes, like Lagoa de São Bras, L. das Furnas or Sete Cidades, suggesting that this species could be more tolerant to degraded ponds than *I. hastata*, although *I. pumilio* is also absent from a few eutrophic ponds, notably in Pico (Table 1). In North America, *I. hastata* has been recorded associated with low levels of nutrients and high levels of oxygen and increased vegetation (Osborn 2005), which suggests that sexual and parthenogenetic strains may have similar ecological requirements, though anecdotal observations suggest that sexual *I. hastata* is more tolerant to eutrophic conditions and colonises marginal habitats that other species have not utilised.

Some of these ponds were sampled repeatedly over the flight season, and the patterns described above remained unchanged. Nevertheless, in two cases, i.e. Lagoa do Gato in Pico and Lagoa do Pau Pique in São Miguel, *I. pumilio* was found to be more common earlier in the year, which could suggest changes in the relative abundance of both species through the season. Lastly, preliminary genetic analyses using microsatellites have detected only one clone in the parthenogenetic populations from the Azores (Lorenzo Carballa et al. 2007), and the genetic mechanism of parthenogenesis in this species has proved to be apomictic, i. e. no meiosis exists during oogenesis, thus leaving no space for the formation of new gene combinations, except by mutation (Lorenzo-Carballa & Cordero-Rivera 2009). This lack of genetic variability could also explain why these parthenogenetic animals are unable to adapt to eutrophic conditions in the ponds.

Further research, including the larval sampling in several eutrophic and oligotrophic ponds, as well as a detailed analysis of the chemicals present in the water of these ponds (see Osborn 2005) is needed to fully understand the ecological requirements of both sexual and parthenogenetic *I. hastata*.

CONCLUSIONS AND IMPLICATIONS FOR CONSERVATION MANAGEMENT

We found that population density of parthenogenetic *I. hastata* colonies in the Azores can be very high, at least in favourable habitats. A relatively high survivorship, high fertility rates, and the lack of both competitors and predators in the ponds could explain these high densities despite the low number of ovipositions recorded. Given the daily estimates of population size, it is likely that the ponds in the archipelago produce more than 100,000 animals per year, thus ensuring the persistence of these parthenogenetic populations, at least in the short term. However, the fact that we found this species associated with oligotrophic ponds, whereas it was apparently absent from eutrophic ponds, especially when eutrophication resulted from degradation by farming activities, suggests that this remarkable species could be at risk on some of the islands. In fact, the species became extinct in at least one pond, the Lagoa do Capitão in Pico, since Belle's visit in 1988 (Belle & van Tol 1990), and is now very rare in Lagoa do Caiado (Table 1). Furthermore, we could not find this species on the island of Graciosa, where all the ponds are strongly eutrophic (Azevedo et al. 2004). The density of cattle in the Azores is very high and the demand for water is increasing, two factors that, together with the proliferation of cyanobacteria due to nutrient inputs to pastures, accelerate eutrophication. Fortunately, there are plans to improve water quality in some lakes, such as Lagoa de Furnas and Sete Cidades in São Miguel (Medeiros et al. 2006), and some ponds are within volcanic craters and thus protected from human influence due to their relative isolation, which should allow for future survival of these truly unique zygopterans, if these habitats remain protected.

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